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Catalytic function and local proton structure at the Type 2 copper of nitrite reductase: The correlation of enzymatic pH dependence, conserved residues, and proton hyperfine structure

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Abstract

Electron nuclear double resonance (ENDOR) of protons at Type 2 and Type 1 cupric active sites correlates with the enzymatic pH dependence, the mutation of nearby conserved, nonligating residues, and electron transfer in heterologously expressed Rhodobacter sphaeroides nitrite reductase. Wild-type enzyme showed a pH 6 activity maximum but no kinetic deuterium isotope effect, suggesting protons are not transferred in the rate-limiting step of nitrite reduction. However, protonatable Asp129 and His287, both located near the Type 2 center, modulated enzyme activity. ENDOR of the wild-type Type 2 center at pH 6.0 revealed an exchangeable proton with large hyperfine coupling. Dipolar distance estimates indicated that this proton was 2.50-2.75 or 2.25-2.45 Å from Type 2 copper in the presence or absence of nitrite, respectively. This proton may provide a properly oriented hydrogen bond to enhance water formation upon nitrite reduction. This proton was eliminated at pH 5.0 and showed a diminished coupling at pH 7.5. Mutations of Asp129 and His287 reduced enzyme activity and altered the exchangeable proton hyperfine spectra. Mutation of Asp129 prevented a pH-dependent change at the Type 1 Cys167 ligand as observed by Cys C β proton ENDOR, implying there is a Type 2 and pH-dependent alteration of the Type 1 center. Mutation of the Type 1 center ligand Met182 to Thr and mutation of Asp129 increased the activation energy for nitrite reduction. Involvement of both the Type 1 center and Asp129 in modulating activation energy shows that electron transfer from the Type 1 center to a nitrite-ligated Type 2 center is rate-limiting for nitrite reduction. Mutation of Ile289 to Ala and Val caused minor perturbation to enzyme activity, but as detected by ENDOR, allowed formate binding. Thus, bulky Ile289 may exclude non-nitrite ligands from the Type 2 active site.

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